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Mediation of the behavioral, endocrine and thermoregulatory actions of ghrelin

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Abstract

The action of ghrelin on telemetrically recorded motor activity and the transmission of the effects of this neuropeptide on spontaneous and exploratory motor activity and some related endocrine and homeostatic parameters were investigated. Different doses (0.5–5 μg) of ghrelin administered intracerebroventricularly caused significant increases in both square crossing and rearing activity in the "open-field" apparatus, while only the dose of 5 μg evoked a significant increase in the spontaneous locomotor activity recorded by telemetry. Ghrelin also induced significant increases in corticosterone release and core temperature. To determine the transmission of these neuroendocrine actions, the rats were pretreated with different antagonists, such as a corticotropin-releasing hormone (CRH) antagonist (α-helical CRH_{9-41}), the nitric oxide synthase inhibitor *N*ω-nitro-L-arginine-methyl ester (L-NAME), haloperidol, cyproheptadine or the cyclooxygenase inhibitor noraminophenazone (NAP). The open-field and biotelemetric observations revealed that the motor responses were diminished by pretreatment with the CRH antagonist and haloperidol. In the case of HPA (hypothalamic pituitary adrenal) activation, only cyproheptadine pretreatment proved effective; haloperidol and L-NAME did not modify the corticosterone response. NAP had only a transient, while cyproheptadine elicited a more permanent impact on the hyperthermic response evoked by ghrelin; the other antagonists proved to be ineffective. The present data suggest that both CRH release and dopaminergic transmission may be involved in the ghrelin-evoked behavioral responses. On the other hand, ghrelin appears to have an impact on the HPA response via a serotonergic pathway and on the hyperthermic response via a cyclooxygenase and a serotonergic pathway.

Keywords: Behavior; HPA axis; Open field; Telemetry

Introduction

In the field of neuroendocrinology, the discovery of ghrelin has been one of the most important achievements of reverse pharmacology (Kojima et al., 1999). It has been identified as the endogenous ligand for a previously known, but orphan G-protein-coupled receptor (Kojima et al., 1999), and the initial experiments disclosed the role it plays in the regulation of feeding (Wren et al., 2000; Nakazato et al., 2001). However, the widespread distribution of ghrelin in the central nervous system (Galas et al., 2002; Cowley et al., 2003) clearly suggests that the peptide should serve as a much more general transmitter in neuroendocrine regulation. Especially the expressions of ghrelin and its receptor in the hypothalamus and the pituitary (Korbonits et al., 2001) point to a prominent role of this neuropeptide in the regulation of behavioral, endocrine and homeostatic processes.

According to previous publications, ghrelin might be an important regulator of not only feeding but other behavioral processes in rodents (Asakawa et al., 2001; Carlini et al., 2002; Tang-Christensen et al., 2004) and in other vertebrates (Matsuda et al., in press).

Therefore, in the first set of the present experiments, we set out to clarify the action of ghrelin and mediation of its effects on spontaneous locomotion by telemetric observations.

Furthermore, earlier works had revealed the intrinsic activity of ghrelin on anxiety-related behavior.

The administration of ghrelin proved to decrease the number of entries into the open spaces and the time spent on the open

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arms in the plus-maze, indicating an anxiogenic effect (Asakawa et al., 2001; Carlini et al., 2002), and increase the latency time in the step-down test (Carlini et al., 2002).

Therefore, we placed a great emphasis on establishing the mediation of the locomotor effects of ghrelin not only on horizontal locomotion, but also on exploratory behavior (square crossing, rearing) with the help of an "open-field" system. Since corticotropin-releasing hormone (CRH) is one of the most potent regulators of spontaneous locomotion and anxiety-related behavior (Monnikes et al., 1992; Menzaghi et al., 1994) and CRH appears to mediate the actions of ghrelin on the HPA system (Asakawa et al., 2001), animals were pretreated with a CRH antagonist (α-helical $CRH_{9–41}$) in order to shed light on the involvement of the CRH transmission in the behavioral effects of ghrelin. As dopamine mediates a number of motor processes in the nigrostriatal and mesolimbic circuitries (Majovski et al., 1981), the nonselective dopamine receptor antagonist haloperidol was administered to scrutinize the mechanisms of ghrelin-dependent motor processes. The serotonin antagonist cyproheptadine was applied as pretreatment for two reasons: serotonin plays a diverse role in the regulation of behavior (Hillegaart, 1990), and it appears to be involved in the action of ghrelin on growth hormone (GH) secretion (Pinilla et al., 2003). Recent data have provided evidence of the role of NO in the mediation of the action of ghrelin in food consumption (Gaskin et al., 2003) and GH secretion (Pinilla et al., 2003). Furthermore, ghrelin proved to have a strong impact on nitric oxide synthase (NOS) activity in the control of pancreatic hormone secretion. NO is likewise a well-established mediator of various motor paradigms evoked by cocaine (Pudiak and Bozarth, 1993) and opiates (Calignano et al., 1993). Accordingly, the effects of pretreatment with the NOS inhibitor *N*ω-nitro-L-arginine-methyl ester (L-NAME) were tested, too.

Previous studies have demonstrated that ghrelin activates the HPA system at a hypothalamic level (Asakawa et al., 2001). The present experiments were designed to extend our understanding of the role of ghrelin in the regulation of the HPA system, focusing on the feasible interactions between ghrelin and the dopaminergic, serotonergic and NO-cGMP systems. While serotonin and ghrelin play opposite roles in the regulation of feeding (Carruba et al., 1986; Nakazato et al., 2001), both appear to activate the HPA system (Fuller, 1990; Asakawa et al., 2001). It therefore seemed especially interesting to elucidate the possible interactions in the regulation of the HPA system, via pretreatment with a serotonin antagonist. Since NO also exerts strong effects on the different levels of the HPA axis (Rivier and Shen, 1994), it promised to be worthwhile to investigate the role of NO in the neuroendocrine response evoked by ghrelin. As previous studies had suggested that dopaminergic mediation might be involved in the HPA response (Jezova et al., 1985), haloperidol was also applied as pretreatment.

The control of food consumption and thermoregulation are strongly interrelated, and numerous neuropeptides, such as CRH (Morley and Levine, 1982; Nakamori et al., 1993), melanocyte-stimulating hormone (Kandasamy and Williams, 1984; Abbott et al., 2000) and neuropeptide Y (NPY) (Currie and Coscina, 1995), have been found to exert effects on both processes. Since ghrelin is a well-established regulator of feeding, in our experiments, we set out to detect only its acute and longer-lasting action on thermoregulation by means of the telemetric observation of core temperature. Moreover, with the preadministration of a nonspecific cyclooxygenase (COX) inhibitor noraminophenazone (NAP), we tested that this action is completely prostaglandin-dependent or might be mediated by other transmitters. The literature indicates that CRH (Strijbos et al., 1992; Nakamori et al., 1993), dopamine, serotonin (Frey, 1975, Oka et al., 2001) and NO (Gourine, 1995) may all mediate thermoregulatory responses, therefore the possible effects of the previously mentioned antagonists on core temperature were also recorded.

Materials and methods

Animals

The animals were kept and handled during the experiments in accordance with the instructions of the University of Szeged Ethical Committee for the Protection of Animals in Research. Male Wistar rats weighing 150–250 g upon arrival were used. The rats were kept in their home cages at a constant room temperature on a standard illumination schedule with 12-h light and 12-h dark periods (lights on from 6:00 a.m.). Commercial food and tap water were available ad libitum. The rats were allowed a minimum of 1 week to acclimatize before surgery. To minimize the effects of nonspecific stress, the rats were handled daily.

Surgery

For intracerebroventricular (icv) peptide administration, the rats were implanted with a stainless steel Luer cannula (10 mm long) aimed at the right lateral cerebral ventricle under Nembutal (35 mg/kg, ip) anesthesia. The stereotaxic coordinates were 0.2 mm posterior; 1.7 mm lateral to the bregma; 3.7 mm deep from the dural surface, according to the atlas of Pellegrino et al. (1979). Cannulas were secured to the skull with dental cement and acrylate. The rats were used after a recovery period of at least 5 days. For implantation of an internal radio transmitter (E-Mitter), the rats were anesthetized with Nembutal (35 mg/kg, ip). The abdomen was opened by making a 2 cm midline incision along the linea alba. The E-Mitter was placed in the abdominal cavity along the sagittal plane in front of the caudal arteries and veins, but dorsal to the digestive organs. The abdominal opening was then closed with absorbable suture material, while the skin was closed with stainless steel suture material.

Treatment

Protocol 1

Different doses of ghrelin (0.5–5 μg) (Bachem, Switzerland) dissolved in saline, or saline alone (control animals), in a volume of 2 μl, were injected icv into conscious rats.

Protocol 2

For this experimental setting, animals were subjected to combined treatment with α-helical $CRH_{9–41}$ (Bachem, Switzerland), the nonselective dopamine receptor blocker haloperidol (Richter, Hungary), the NOS inhibitor L-NAME (Sigma, Hungary), the serotonin antagonist cyproheptadine (Tocris, UK) or the pyrazolone derivative noraminophenazone (NAP) (Algopyrin; Chinoin, Hungary) and ghrelin. The doses of the antagonists and inhibitors were the concentration that had abolished the action of a given neuropeptide in our previous experiments but per se do not affect the neuroendocrine paradigms (Telegdy, 1984; Bujdoso et al., 2003; Jaszberenyi et al., 2004), while the dose of ghrelin was the concentration that had proved to be the most effective in protocol 1. The doses were the following: α-helical $CRH_{9–41}$: 1 μg (Jaszberenyi et al., 2004); haloperidol: 10 μg/kg (Bujdoso et al., 2003; Jaszberenyi et al., 2004); L-NAME: 20 μg (Jaszberenyi et al., 2004); NAP: 50 mg/kg (Jaszberenyi et al.,